PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Marco CATTARUZZA and
Markus HECKER

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For: FUNCTIONAL CORRECTION OF THE 786C/T-VARIANCE OF THE HUMAN eNOS-GENE

Group Art Unit: 1635

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CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

April 13, 2007
Date

Steven L. Highlander

DECLARATION OF MARKUS HECKER UNDER 37 C.F.R. §1.132

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

I, Dr. Markus Hecker, do declare that:

1. I am a citizen of Germany residing at Heidelberg. I currently hold the position of Full Professor and Chairman at the Institute of Physiology and Pathophysiology of the University Hospital Heidelberg. My research experience includes well over 100 original articles in peer reviewed international scientific journals and close to 40 review articles in scientific journals, journal supplements, conference proceedings and books. I have

trained in Biology, Biochemistry, Pharmacology and Physiology and hold several university degrees including a doctorate in Biochemistry and a state doctorate in Physiology. I have worked in cardiovascular research for almost 20 years, mainly focusing on molecular and cell biology issues in vascular cells. I have a special expertise in the analysis and therapeutic manipulation of transcription factors and in this capacity have been the inventor of 69 patent applications of which 9 have been granted. A copy of my *curriculum vitae* is appended hereto.

2. Attached as Exhibit A are illustrations regarding the cellular uptake of decoy oligodeoxyribonucleotides (ODNs) according to the present invention. Pages 2-4 of Exhibit A show visualization of fluorescent-labeled decoy ODNs in the cytosol and nucleus of human vascular endothelial cells (HUVEC) by fluorescence microscopy and laser-scanning microscopy (LSM). Diffuse and particulate cytosolic fluorescence might indicate different uptake mechanisms. Counterstaining with an antibody against the surface receptor CD31 shows intracellular localization of decoy ODNs. Uptake of the decoy ODNs is concentration and time dependent, with a maximal uptake observed at 10 μM decoy ODN after approximately 1 hr incubation.

3. Pages 6 and 7 of Exhibit A show the kinetics of cellular uptake using ^{35}S-labeled decoy ODNs at 10 μM and increased incubation times revealed maximal cytosolic radioactivity, as determined by scintillation counting of cell lysate, after approximately 1 to 2 hrs incubation. A rough estimate of the intracellular concentration at this peak yields

approximately 30 μM decoy ODN. Thereafter, a steady decline of cytosolic radioactivity to equilibrium level of 10 μM decoy ODN was observed over 48 hrs.

4. Exhibit A, pages 9, 10, 12 and 13 show that the cellular uptake of short, double-stranded DNA decoy ODNs appears to be mediated by the folate transport mechanisms of the cell, *i.e.*, the reduced folate carrier (hRFC) and the folate receptor (FR). Decoy ODN uptake shows pharmacological characteristics regarding chloride concentration and pH of the medium that resemble the characteristics of the hRFC. Further, a more than 60% reduction in hRFC expression due to antisense inactivation resulted in a 40% reduced cellular uptake of a fluorescent labelled decoy ODN. In addition, competitive inhibition of the cellular folate transport mechanisms by folate or folate analog (methotrexate) yields a substantially reduced cellular uptake of short double-stranded decoy ODNs. In comparison, the hRFC appears to operate much less efficient for the uptake of single-stranded antisense DNA or double-stranded siRNA molecules.

5. Pages 15-17 of Exhibit A show that short, double-stranded decoy ODNs efficiently penetrate into psoriatic skin. *In vitro* application of a 2% ointment formulation of a fluorescent-labelled decoy ODN on psoriatic skin biopsies showed a deep penetration of the decoy ODN beyond the basal membrane into the dermal layer of the skin. Using direct fluorescence or anti-FITC immunohistochemistry of skin sections, nuclear staining of decoy ODN in keratinocytes within the epidermis and infiltrating inflammatory cell clusters in the dermis was observed.

6. Pages 19-22 of Exhibit A show that short, double-stranded decoy ODNs efficiently enter bronchial epithelium. Intranasal application of a fluorescent-labeled decoy ODN in mice led to a bright fluorescence of bronchial epithelium 10 min after application. Cellular uptake into bronchial epithelial cells is not different in normal, untreated mice as compared to mice with an airway inflammation induced by treatment with allergen.

7. I declare that all statements made herein of my own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under § 1001 of title 18 of the United States Code.

Heidelberg, 04-13-2007

Date

Dr. Markus Hecker

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Born:	04 January 1960

Scientific curriculum

1980-1985	Study of Biology at the University of Konstanz, Germany
1985	Diploma (M. Sc. in Biology), University of Konstanz
1985-1987	Postgraduate studies at the University of Konstanz
1988	Dr. rer. nat. (Ph. D. in Biochemical Pharmacology), University of Konstanz
1988-1989	Visiting scientist, Department of Physiology and Biophysics, Georgetown University, Washington, D.C., U.S.A
1989-1990	Visiting scientist, William Harvey Research Institute, St. Bartholomew's Hospital Medical College, London, U.K.
1990-1991	Senior Scientist and Honorary Lecturer, William Harvey Research Institute, London
1991-1993	Lecturer, Department of Applied Physiology, University of Freiburg, Germany
1993	State doctorate (Dr. rer. nat., habil. in Physiology), University of Freiburg
1993-1996	Assistant Professor, Department of Cardiovascular Physiology, University of Frankfurt/M., Germany
1996-2004	Professor (C3) and Head, Department of Cardiovascular Physiology, University of Göttingen, Germany
2004 –	Professor (C4) and Director, Institute of Physiology and Pathophysiology, University of Heidelberg, Germany
2006 –	Head of the Division of Cardiovascular Physiology and Managing Director of the Institute of Physiology and Pathophysiology, University of Heidelberg

Honors

1987-1988	Post-graduate scholarship, Boehringer Ingelheim Fonds
1988-1990	Post-doctoral fellowship, German Research Foundation (DFG)
1991-1993	Lecturer fellowship, German Research Foundation (DFG)
1993	Sandoz Award for Therapy-Related Pharmacological Research, German Society of Experimental and Clinical Pharmacology and Toxicology
1994-1996	Heisenberg fellowship, German Research Foundation (DFG)
2000	Wulf Vater Dihydropyridine Research Award, Wulf Vater-Foundation

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